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1. Document ID: US 6569035 B2

L7: Entry 1 of 15 File: USPT May 27, 2003

US-PAT-NO: 6569035
DOCUMENT-IDENTIFIER: US 6569035 B2

TITLE: Golf ball comprising silicone material

DATE-ISSUED: May 27, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Binette; Mark L.	Ludlow	MA		
Sullivan; Michael J.	Barrington	RI		

US-CL-CURRENT: 473/373

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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2. Document ID: US 6508724 B2

L7: Entry 2 of 15 File: USPT Jan 21, 2003

US-PAT-NO: 6508724
DOCUMENT-IDENTIFIER: US 6508724 B2

TITLE: Golf ball cores with improved durability

DATE-ISSUED: January 21, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Dalton; Jeffrey L.	Dartmouth	MA	02747	

US-CL-CURRENT: 473/367; 264/236

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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3. Document ID: US 6392024 B1

L7: Entry 3 of 15 File: USPT May 21, 2002

US-PAT-NO: 6392024
DOCUMENT-IDENTIFIER: US 6392024 B1

TITLE: Tenebrio antifreeze proteins

DATE-ISSUED: May 21, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Graham; Laurie A.	Kingston			CA
Liou; Yih-Cherng	Kingston			CA
Walker; Virginia K.	Sydenham			CA
Davies; Peter L.	Kingston			CA

US-CL-CURRENT: 536/23.5; 435/252.3, 435/254.11, 435/254.21, 435/254.22, 435/320.1, 435/6, 536/23.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. Desc | Image

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☐ 4. Document ID: US 6348569 B1

L7: Entry 4 of 15 File: USPT Feb 19, 2002

US-PAT-NO: 6348569
DOCUMENT-IDENTIFIER: US 6348569 B1

TITLE: Spruce budworm antifreeze proteins, genes and method of using same

DATE-ISSUED: February 19, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Walker; Virginia K.	Sydenham			CA
Davies; Peter L.	Kingston			CA
Rahavard; Mitra	Kingston			CA
Tyshenko; Michael G.	Kingston			CA

US-CL-CURRENT: 530/300; 530/350

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

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☐ 5. Document ID: US 6332850 B1

L7: Entry 5 of 15 File: USPT Dec 25, 2001

US-PAT-NO: 6332850
DOCUMENT-IDENTIFIER: US 6332850 B1

TITLE: Golf ball cores with improved durability

DATE-ISSUED: December 25, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Dalton; Jeffrey L.	Dartmouth	MA		

US-CL-CURRENT: 473/371; 473/373, 473/374, 473/376

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments   KWIC | Draw Desc | Image

6. Document ID: US 6303388 B1

L7: Entry 6 of 15   File: USPT   Oct 16, 2001

US-PAT-NO: 6303388
DOCUMENT-IDENTIFIER: US 6303388 B1

TITLE: Process for preparing novel ice-controlling molecules

DATE-ISSUED: October 16, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Fahy; Gregory M.	Gaithersburg	MD		

US-CL-CURRENT: 436/518; 252/70, 424/184.1, 435/7.1, 435/7.8

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments   KWIC | Draw Desc | Image

7. Document ID: US 6162134 A

L7: Entry 7 of 15   File: USPT   Dec 19, 2000

US-PAT-NO: 6162134
DOCUMENT-IDENTIFIER: US 6162134 A

TITLE: Low spin golf ball comprising silicone material

DATE-ISSUED: December 19, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Sullivan; Michael J.	Chicopee	MA		
Nesbitt; R. Dennis	Westfield	MA		
Binette; Mark L.	Ludlow	MA		

US-CL-CURRENT: 473/373; 473/354, 473/363, 473/364, 473/365, 473/374, 473/375, 473/376, 473/377, 473/378

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments   KWIC | Draw Desc | Image

8. Document ID: US 6120390 A

L7: Entry 8 of 15   File: USPT   Sep 19, 2000

US-PAT-NO: 6120390
DOCUMENT-IDENTIFIER: US 6120390 A

TITLE: Golf ball cores with improved durability

DATE-ISSUED: September 19, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Dalton; Jeffrey L.	Dartmouth	MA		

US-CL-CURRENT: 473/351; 473/358, 473/365, 473/371, 473/372, 473/373, 473/374, 473/377, 473/378, 473/385

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments   KWIC | Draw Desc | Image

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9. Document ID: US 6008016 A

L7: Entry 9 of 15   File: USPT   Dec 28, 1999

US-PAT-NO: 6008016
DOCUMENT-IDENTIFIER: US 6008016 A

TITLE: Spruce budworm antifreeze proteins, genes and methods of using same

DATE-ISSUED: December 28, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Walker; Virginia K.	Sydenham			CA
Davies; Peter L.	Kingston			CA
Rahavard; Mitra	Kingston			CA
Tyshenko; Michael G.	Kingston			CA

US-CL-CURRENT: 435/69.1; 435/252.3, 435/252.33, 435/254.11, 435/254.2, 435/254.21, 435/320.1, 435/325, 435/410, 530/300 , 530/350, 536/23.5

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments   KWIC | Draw Desc | Image

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10. Document ID: US 5925676 A

L7: Entry 10 of 15   File: USPT   Jul 20, 1999

US-PAT-NO: 5925676
DOCUMENT-IDENTIFIER: US 5925676 A

TITLE: Ester compounds pesticidal compositions containing the same and itermediates for synthesis thereof

DATE-ISSUED: July 20, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Iwasaki; Tomonori	Sanda			JP
Tsushima; Kazunori	Sanda			JP
Sugano; Masayo	Sanda			JP

US-CL-CURRENT: 514/531; 514/445, 514/473, 514/519, 514/521, 514/532, 549/323, 549/66, 558/404, 558/434, 560/105, 560/118 , 560/124, 560/55, 560/8

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments   KWIC | Draw Desc | Image

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L7: Entry 4 of 15 File: USPT Feb 19, 2002

DOCUMENT-IDENTIFIER: US 6348569 B1
TITLE: Spruce budworm antifreeze proteins, genes and method of using same

Abstract Text (1):
A novel class of thermal hysteresis, antifreeze proteins (THPs) has been isolated and purified from Choristoneura sp., including the eastern spruce budworm C. fumiferana. The invention provides for nucleic acids which encode these antifreeze proteins. The invention also provides for antibodies reactive to these novel antifreeze proteins. The invention also includes a method for decreasing the freezing point of an aqueous solution by adding these novel antifreeze proteins to the solution.

Brief Summary Text (3):
In the modern world, frozen foods have become a mainstay of the human diet. To ensure a high quality product, sufficient for the demanding consumer's palate, frozen vegetables in particular, and frozen desserts, such as ice cream, have been the subject of extensive research by food processors. It is now known that recrystallization can have a substantial negative impact on the taste and texture of frozen foods. The advent of frost-free freezers has exacerbated this situation, which has been more traditionally associated with temperature fluctuations during transportation. After a relatively short period of time at other than sub-zero temperatures or even at sustained freezing temperatures, many frozen foods become less desirable, or worse, totally unsuitable, for human consumption.

Brief Summary Text (4):
While a variety of techniques have been implemented to mitigate the damages associated with recrystallization, and limited success has been attained, significant problems remain. Often, modifications to the processing of the frozen foods drastically affect their quality, color, flavor, and/or texture. Moreover, the additional processing can be very expensive and time consuming, rendering the techniques uneconomical. Similar difficulties have been associated with incorporating additives to the foodstuffs.

Brief Summary Text (6):
Although the first description of protein-mediated thermal hysteresis (TH) was noted in Tenebrio molitor approximately 30 years ago (Grimstone, et al., Philos. Trans. B 253:343 (1968)), numerous attempts to purify these thermal hysteresis proteins (THP) failed to yield pure fractions with enough TH to account for the hemolymph activity (Grimstone, et al., (1968); Patterson & Duman, J. Exp. Zool. 210:361 (1979); Schneppenheim & Theede Comp. Biochem. Physiol. 67B:561 (1980); Tomchaney, et al., Biochemistry 21:716 (1982); Paterson Duman J. Exp. Zool. 219:381 (1982); and (Horwath, et al., Eur. J. Entomol. 93: 419 (1996)). Homogeneity of these proteins was not proven, and they differed in amino acid composition from each other and from the compositions reported here.

Brief Summary Text (9):
The eastern spruce budworm, Choristoneura fumiferana, and western spruce budworm, Choristoneura occidentalis, are freeze-tolerant pests of North American forests. A defense against freezing is the thermal hysteresis (TH) activity of their hemolymph, which allows the insects to depress their freezing points in the presence of ice or ice nucleators. This activity is quantified as the temperature difference (.degree.C.) between the freezing and melting points of a solution containing ice. Hemolymph from spruce budworm larvae demonstrates a freezing point depression of

greater than 4.degree. C.

Brief Summary Text (10):
This invention provides for the nucleic acid molecules that encode the proteins responsible for the thermal hysteresis in Choristoneura larvae, such as the nucleic acid of SEQ ID NO:1. The invention also provides for an isolated nucleic acid encoding an antifreeze protein where the protein can be defined as follows: having a calculated molecular weight of between 7 and 15 kDa; having a thermal hysteresis activity greater than about 1.5.degree. C. at a concentration of about 1 mg/mL; and, specifically binding to an antibody raised against antifreeze proteins or antigenic fragments thereof selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:3, or, having at least 60% amino acid sequence identity to an antifreeze protein selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:3. In a preferred embodiment, the isolated nucleic acid encodes a protein with a calculated molecular weight between about 8 and 12 kDa. In a preferred embodiment, the nucleic acid encodes a protein with a thermal hysteresis activity that is greater than 2.degree. C. at a concentration of about 1 mg/mL. In a preferred embodiment, the nucleic acid encodes a protein with at least 80% sequence identity to an antifreeze protein selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:3. In a further embodiment, the invention provides for a nucleic acid encoding a protein with the sequence listed in SEQ ID NO:2 and SEQ ID NO:3. In another embodiment, the isolated nucleic acid encodes an antifreeze protein found in insects, and in a preferred embodiment that insect is a Choristoneura sp. The invention also provides for an isolated nucleic acid which specifically hybridizes to SEQ ID NO:1 under stringent conditions. The invention further provides for an isolated nucleic acid from a purified Choristoneura sp. antifreeze protein which specifically binds to an antibody directed against antifreeze proteins selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:3.

Detailed Description Text (5):
The THPs of the invention are more active than any purified antifreeze protein published to date. The freezing point depression provided by the THPs of the invention are several times greater than that provided by fish AFPs (THPs) or by T. molitor THP as prepared by Patterson, as described in Patterson, J., et al., The role of the thermal hysteresis factor in Tenebrio molitor larvae, J. Exp. Biol. 74: 37-45 (1978).

Detailed Description Text (6):
However, like fish antifreeze proteins, Choristoneura THP appears to act by an adsorption-inhibition mechanism. Ice crystals in the presence of THP stop growing until the non-equilibrium freezing point is exceeded. At that point, the ice crystals burst forth from the crystal nucleator to form a solid mass of ice principally along the a-axis and the ice fronts are broad and smooth. In contrast, once the freezing point is exceeded in the presence of fish AFPs, myriad ice spicules burst out along and parallel to the c-axis.

Detailed Description Text (14):
The term "antifreeze protein" refers to a protein found in the body fluids of some poikilothermic organisms, such as Choristoneura sp. such as C. fumiferana or C. occidentalis, the Tenebrio molitor mealworm and plants which have the commonly known property that they reduce non-colligatively the freezing point of water. THP are also known as "thermal hysteresis proteins." As used herein, "antifreeze proteins" or "THP" includes chemically synthesized and recombinantly produced polypeptides having a protein sequence with substantial similarity to a naturally occurring antifreeze protein and retaining the properties of an antifreeze polypeptide.

Detailed Description Text (17):
In addition to inspecting visually ice crystal formation, a thermal hysteresis assay can measure the difference between the freezing and melting points of a solution. The melting point of a solution is the temperature at which there is only one ice crystal left in a solution (see, infra, for a more complete description of TH activity).

Detailed Description Text (29):
"Stringent hybridization" and "stringent hybridization wash conditions" in the

context of nucleic acid hybridization experiments such as Southern and northern hybridizations are sequence dependent, and are different under different environmental parameters. An extensive guide to the hybridization of nucleic acids is found in Tijssen (1993) LABORATORY TECHNIQUES IN BIOCHEMISTRY AND MOLECULAR BIOLOGY--HYBRIDIZATION WITH NUCLEIC ACID PROBES part I chapter 2 "overview of principles of hybridization and the strategy of nucleic acid probe assays", Elsevier, N.Y. Generally, highly stringent hybridization and wash conditions are selected to be about 5.degree. C. lower than the thermal melting point (T.sub.m) for the specific sequence at a defined ionic strength and ph. The T.sub.m is the temperature, under defined ionic strength and pH, at which 50% of the target sequence hybridizes to a perfectly matched probe. Very stringent conditions are selected to be equal to the T.sub.m for a particular probe. An example of stringent hybridization conditions for hybridization of complementary nucleic acids which have more than 100 complementary residues on a filter in a Southern or northern blot is 50% formalin with 1 mg of heparin at 42.degree. C., with the hybridization being carried out overnight. An example of highly stringent wash conditions is 0.15 M NaCl at 72.degree. C. for about 15 minutes. An example of stringent wash conditions is a 0.2.times.SSC wash at 65.degree. C. for 15 minutes (see Sambrook for a description of SSC buffer). Often, a high stringency wash is preceded by a low stringency wash to remove background probe signal. An example medium stringency wash for a duplex of, e.g., more than 100 nucleotides, is 1.times.SSC at 45.degree. C. for 15 minutes. An example low stringency wash for a duplex of, e.g., more than 100 nucleotides, is 4-6.times.SSC at 40.degree. C. for 15 minutes. In general, a signal to noise ratio of 2.times. (or higher) than that observed for an unrelated probe in the particular hybridization assay indicates detection of a specific hybridization. Nucleic acids which do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides which they encode are substantially identical. This occurs, e.g., when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code.

Detailed Description Text (30):
The term "thermal hysteresis activity" or "TH activity" refers to the ability to alter the temperature difference (.degree. C.) between the freezing and melting points of a solution containing ice. Preferably, TH activity is be measured by observation of ice crystal formation in a nanoliter osmometer following the procedure set forth in Lawson & Semler, Proc. Nat'l Acad. Sci. USA 88:9919 (1991). Alternatively, TH activity can be determined according to the method described in deVries, METHODS IN ENZYMOLOGY, VOL. 127, Packer (ed.), Academic Press, New York (1986) or a variation thereof.

Detailed Description Text (84):
In addition to bacterial expression systems, the THP of this invention can be expressed in other systems, in particular yeast and baculovirus, but also in mammalian and plant cells. The system used will depend on the lack of success in other systems, the ability to fold the THP properly, and the eventual use of the THP. For example, if the THP are to be used to protect bread dough yeast from freezing (see, U.S. Pat. No. 5,118,792), a yeast system will be used. If plants which can live through freezing temperatures are desired, transgenic techniques can be used to make transgenic plants. However, of course, the system used should give THP with comparable thermal hysteresis activity to that found in Choristoneura sp. larvae.

Detailed Description Text (101):
By the assays described below, the THP of this invention share characteristics with a thermal hysteresis protein isolated from second larval instar Choristoneura fumiferana and Choristoneura occidentalis hemolymph. These assays are used to define whether other novel THP are sufficiently related to these prototype proteins so as to fall within the scope of this invention. The assays can also be pursued to detect and quantify THP proteins present in bacteria broth, tissue culture fluid and plant and animal tissues.

Detailed Description Text (122):
A hapten inhibition assay is another preferred competitive assay. In this assay a known analyte, in this case THP, is immobilized on a solid substrate. A known amount of anti-THP antibody is added to the sample, and the sample is then contacted with

the immobilized THP. In this case, the amount of anti-THP antibody bound to the immobilized THP is inversely proportional to the amount of THP present in the sample. Again the amount of immobilized antibody may be detected by detecting either the immobilized fraction of antibody or the fraction of the antibody that remains in solution. Detection may be direct where the antibody is labeled or indirect by the subsequent addition of a labeled moiety that specifically binds to the antibody as described above.

Detailed Description Text (160):
The THP species and isoforms of the invention can be identified and characterized by at least two functional properties, for example, thermal hysteresis and unique formation of ice crystals:

Detailed Description Text (161):
(1) Thermal Hysteresis

Detailed Description Text (162):
The THP proteins of this invention are approximately 10 to 50 fold more active than known fish (AFP) antifreeze proteins in a thermal hysteresis assay. Thermal hysteresis is defined as the difference between the solution freezing and melting temperatures. Freezing point is taken as the temperature at which uncontrollable crystal growth or spicular ice growth grows. Melting point is taken as the warmest temperature at which an ice crystal can be stably held without melting. TH activity can be measured in a nanoliter osmometer (Clifton Technical Physics, Martford, N.Y.) by methods well known in the art (see, for example. Chakrabartty & Hew, Eur. J. Biochem. 202:1057 (1991)). The starting ice crystal is usually 20 to 50 .mu.m in diameter. The buffer used is typically 100 mM NH.sub.4 HCO.sub.3 (pH 7.9) but other buffers of similar osmolarity can be used. Alternatively, TH activity can be measured in bacterial broth, tissue culture fluid, or hemolymph. However, the TH values obtained will depend on the osmolarity of the solution being measured.

Detailed Description Text (163):
The osmometer is a thermal electric cooling module with a separate but linked variable temperature control. This apparatus allow temperature regulation in the 0.degree. C. to -9.degree. C. range with a deep freeze mode to -40.degree. C. The cooling module can be set up on a microscope stage where the growth and melt behavior of an ice crystal can be observed directly. The sample holder can be a small plate with dimensions of about 7 mm.times.7 mm.times.0.75 mm containing multiple small sample holes (about 0.35 mm in diameter). A drop of immersion oil, such as Cargille's B immersion oil, can be placed on the underside of the sample holder so that the sample holes are filled. 1 to 5 nL samples are then delivered into the center of the oil-filled hole by a capillary tube.

Detailed Description Text (164):
To measure TH activity, the samples are first frozen by cooling the samples rapidly to -40.degree. C. and then allowing them to warm up to the melting point temperature. Once the melting point is reached, the samples are cooled by approximately 0.02.degree. C. (10 milli-osmoles or mosmoles) per 10 to 15 seconds until the freezing temperature is reached. The conversion from the unit "osmos" to ".degree. C." is 1.00 osmoles equals 1.86.degree. C. In most instances, when the freezing point of a THP sample is reached, the ice crystal within the sample will grow spontaneously and rapidly. This leads to freezing of the entire sample.

Detailed Description Text (165):
Alternatively, a small ice crystal can be frozen onto the surface of a solution and the temperature of the solution immediately reduced to below freezing. The temperature below freezing when the nucleated ice crystal begins to grow is the freezing point depression or the thermal hysteresis measurement (see, Patterson & Duman, J. Exp. Zool. 210:361 (1979); and Wu, et al., J. Comp. Physiol. B 161:271 (1991). The thermal hysteresis activity of a solution is dependent on the concentration of the antifreeze protein, with the greater the concentration of the protein, the greater the activity shown by the solution. However, increased concentrations of THPs produce incrementally smaller increases in TH activity and a maximum is approached. In other words, the relationship between THP concentration and TH is hyperbolic, not linear.

Detailed Description Text (166):
The proteins of the present invention preferably have thermal hysteresis values greater than about 1.5.degree. C. at about 1 mg/mL, more preferably greater than 2.degree. C. at about 1 mg/mL and most preferably between about 1.5.degree. C. to 3.0.degree. C. at about 1 mg/mL.

Detailed Description Text (195):
Antifreeze activity was assayed during extraction and purification of THP by thermal hysteresis (i.e., the difference in temperature (.degree.C.) between the freezing and melting points of a solution) using a Clifton Direct Reading Nanoliter Osmometer (Clifton Technical Physics, Hartford, N.Y.) according to the method of Chakrabartty et al. (1991). During these assays the effects of the AFP on ice crystal morphology were monitored and recorded by video microscopy. Total protein concentration was monitored by colorimetric assays (Bradford, 1976) or by absorbance at 230 nm (A.sub.230).

Detailed Description Text (197):
THPs were purified from the resulting filtrate using chromatographic procedures that are standard for the isolation of fish AFPs from serum (Fourney et al., 1984; Li et al., 1985; Ng et al., 1986). First, the THPs were size-selected by gel filtration at 4.degree. C. on a Sephadex G-75 column (Pharmacia, Uppsala, Sweden) in the homogenization buffer. The THPs eluted just after the void peak. The fractions with highest thermal hysteresis activity were then pooled, lyophilized, suspended in 1 mL H.sub.2 O, relyophilized and resuspended in 100 .mu.L H.sub.2 O. The sample was next subjected to high performance liquid chromatography (HPLC) (Beckman) on a C18 reversed-phase column in 0.1% trifluoroacetic acid (TFA) using a linear acetonitrile gradient varying from 0 to 65% (v/v). As shown in FIG. 1, THPs eluted from this column as a cluster of peaks, labelled 22, 23 and 24, which demonstrated thermal hysteresis activity. This was consistent with the fractionation of a set of THP isoforms.

Detailed Description Text (208):
PCR can be performed on the cDNA using, for example, a Techne PHC-3 thermal cycler (Techne, Princeton, N.J.) using any set of primers whose sequence is based on a known THP sequence, such as SEQ ID NO:1, or pairs of primers that are known to amplify THP sequences, such as SEQ ID NO:4 and SEQ ID NO:5, and, SEQ ID NO:13 and SEQ ID NO:14.

Detailed Description Text (216):
As described in Example 1, thermal hysteresis assays of THPs were recorded by video microscopy. This led to the following interesting observation: When a solution containing the THP of the invention does freeze, the ice forms smooth waves or fronts. This is shown in FIG. 3A, a photomicrograph of ice formed from a crude extract of second instar larvae of spruce budworm. In contrast, when a solution containing a fish THP freezes, the ice is typically sharp and spicular. An example of this, ocean pout Type III antifreeze protein (5 mg/mL), is shown in FIG. 3B. Such sharp ice formations could cause shearing of cells.

Detailed Description Paragraph Table (1):
SEQUENCE LISTING (1) GENERAL INFORMATION: (iii) NUMBER OF SEQUENCES: 17 (2) INFORMATION FOR SEQ ID NO: 1: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 1387 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: cDNA (ix) FEATURE: (A) NAME/KEY: CDS (B) LOCATION: 65..391 (D) OTHER INFORMATION: /product= "THP precursor" /note= "spruce budworm (Choristoneura sp.) thermal hysteresis protein (THP) precursor" (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1 CGGCACGAGG AAAGACATAT TTTTTTTTTA GTTTCAAAAG TTGTGTACAT TTTTCTCAAG 60 TATC ATG AAG TGT TTA ATG CTG ATC ATG GCT CTA GCC ATT ATC AAC ACT 109 Met Lys Cys Leu Met Leu Ile Met Ala Leu Ala Ile Ile Asn Thr 1 5 10 15 GTA TCT TCT GAT GGC TCG TGT ACA AAC ACG AAC TCT CAG CTC AGC GCA 157 Val Ser Ser Asp Gly Ser Cys Thr Asn Thr Asn Ser Gln Leu Ser Ala 20 25 30 AAC TCC AAG TGC GAA AAA TCG ACG TTG ACC AAC TGC TAC GTC GAT AAA 205 Asn Ser Lys Cys Glu Lys Ser Thr Leu Thr Asn Cys Tyr Val Asp Lys 35 40 45 AGC GAG GTT TAC GGC ACT ACC TGT ACA GGA AGC CGA TTC GAC GGA GTC 253 Ser Glu Val Tyr Gly Thr Thr Cys Thr Gly Ser Arg Phe Asp Gly Val 50 55 60 ACT ATA ACG ACT TCA ACA TCT ACC GGT TCA CGT ATT TCA GGC CCT GGA 301 Thr Ile Thr Thr Ser Thr Ser Thr Gly Ser Arg Ile Ser Gly

Pro Gly 65 70 75 TGC AAG ATT TCC ACT TGC ATT ATC ACC GGG GGT GTA CCT GCT CCA TCA 349 Cys Lys Ile Ser Thr Cys Ile Ile Thr Gly Gly Val Pro Ala Pro Ser 80 85 90 95 GCT GCT TGC AAG ATT TCT GGA TGT ACT TTC AGT GCT AAT TAAGCCATGA 398 Ala Ala Cys Lys Ile Ser Gly Cys Thr Phe Ser Ala Asn 100 105 AAGTCGTCCG AGATTGAGTT TGGCCATTTC ATATGTAAGT AGAATAGGCT AGTGGCTTAA 458 AAAATGTAAT GAGTCCCGTC AGTTAGAATA TCAAAAAACA TGTATTTTTT CGGTTACCTA 518 TATAATGATT CGCCGACAAT TCTTACGCAG ATTATTGATT GGCAGACAAC GTTTCGCCGA 578 GTAAAGGTAC GCTGATGAAT TTGAGCGCAT ATGTATTGTT TCGCGTACTA ACGTTTAATT 638 GACTATTTAT TCATTCAGTT GGCTTATGGG TCAGTAAGCC GAGCAACTAA TAACAGAAAT 698 TCGTTTAGCC GATTAATCAC TACACATTTT GAACGTTTAC TCAAACAAGT TTTCGCTTTA 758 GAATTTGTGA TTATTTTAAA TTTAAAGTCA AAACAAACCA CGTCGCGACA CGCCAGCAGT 818 TCGGTTATTG TTTGCCGCAA TTCTACCTAA CACTCCTCCT CGCTGACGCT CGTCGTTGCA 878 CCTAACTATA TTTCGGTGCC ATGTGATAAC TCTGCTTATC ATGTTCCGGC GAACAGTTGT 938 TCGCTTAACT CAAACAATTA CGAACCAAAC AATCGAATAA ACGTAAATCT GCATACCGCA 998 ATTCTTTCGT ACCGACTACT CGGCGAAATG AATAATAGGC GTAACAATAT TATACCAAAC 1058 GTTGCTCGGC CAAAAGAAAA ATCTGCGTAA TCAAACTCGG CGAATCGACC GGTCACCATT 1118 ATCACTGAAA TAGATGGCCG TAAATTGTAA TCTATTAATT TAATCGATTA ACATGTTTAT 1178 AATAGAATAA TAAATATTAC TTAACATTAC TTAGTATTAA ATGATAGTAA CATATTTTAA 1238 CACTAGAGGG CTAGAAAATT AAAATAAAGC TTACATTATG CTACTCTAAT GACGGACTAA 1298 AAAGATTTTT TTTCCCCAAT TACCACTGTA CTTACTGTTT TTAAATATTT TAAAATACAG 1358 AAATTGTAAC CAAAAAAAAA AAAAAAAAA 1387 (2) INFORMATION FOR SEQ ID NO: 2: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 108 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: protein (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2 Met Lys Cys Leu Met Leu Ile Met Ala Leu Ala Ile Ile Asn Thr Val 1 5 10 15 Ser Ser Asp Gly Ser Cys Thr Asn Thr Asn Ser Gln Leu Ser Ala Asn 20 25 30 Ser Lys Cys Glu Lys Ser Thr Leu Thr Asn Cys Tyr Val Asp Lys Ser 35 40 45 Glu Val Tyr Gly Thr Thr Cys Thr Gly Ser Arg Phe Asp Gly Val Thr 50 55 60 Ile Thr Thr Ser Thr Ser Thr Gly Ser Arg Ile Ser Gly Pro Gly Cys 65 70 75 80 Lys Ile Ser Thr Cys Ile Ile Thr Gly Gly Val Pro Ala Pro Ser Ala 85 90 95 Ala Cys Lys Ile Ser Gly Cys Thr Phe Ser Ala Asn 100 105 (2) INFORMATION FOR SEQ ID NO: 3: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 90 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: (D) TOPOLOGY: linear (ii) MOLECULE TYPE: protein (ix) FEATURE: (A) NAME/KEY: Protein (B) LOCATION: 1..90 (D) OTHER INFORMATION: /note= "mature THP" (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3 Asp Gly Ser Cys Thr Asn Thr Asn Ser Gln Leu Ser Ala Asn Ser Lys 1 5 10 15 Cys Glu Lys Ser Thr Leu Thr Asn Cys Tyr Val Asp Lys Ser Glu Val 20 25 30 Tyr Gly Thr Thr Cys Thr Gly Ser Arg Phe Asp Gly Val Thr Ile Thr 35 40 45 Thr Ser Thr Ser Thr Gly Ser Arg Ile Ser Gly Pro Gly Cys Lys Ile 50 55 60 Ser Thr Cys Ile Ile Thr Gly Gly Val Pro Ala Pro Ser Ala Ala Cys 65 70 75 80 Lys Ile Ser Gly Cys Thr Phe Ser Ala Asn 85 90 (2) INFORMATION FOR SEQ ID NO: 4: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 32 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: DNA (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4 CATATGCATA TGGATGGCTC GTGTACAAAC AC 32 (2) INFORMATION FOR SEQ ID NO: 5: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 33 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: DNA (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5 AAGCTTAAGC TTTTAATTAG CACTGAAAGT ACA 33 (2) INFORMATION FOR SEQ ID NO: 6: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 15 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6 Phe Asp Gly Val Thr Ile Thr Ser Ser Thr Ser Thr Gly Ser Arg 1 5 10 15 (2) INFORMATION FOR SEQ ID NO: 7: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 22 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7 Ser Thr Leu Thr Asn Cys Tyr Val Asp Lys Ser Glu Val Tyr Gly Thr 1 5 10 15 Thr Cys Thr Gly Ser Arg 20 (2) INFORMATION FOR SEQ ID NO: 8: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 18 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8 Ile Ser Ser Cys Ile Ile Thr Gly Gly Val Pro Ala Pro Ser Ala Ala 1 5 10 15 Cys Lys (2) INFORMATION FOR SEQ ID NO: 9: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 25 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9 Asp Gly Thr Xaa Thr Asn Thr Asn Ser Gln Leu Ser Ala Asn Ser Gln 1 5 10 15 Xaa Asp Lys Ser Thr Leu Thr Asn Xaa 20 25 (2) INFORMATION FOR SEQ ID NO: 10: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 19 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10 Asp Gly Thr Xaa Arg Asn Thr Asn Ser Gln Ile Thr Asn Ser Gln Gly 1 5 10 15 Xaa Asp Arg (2) INFORMATION FOR SEQ ID NO: 11: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 18 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11 Met Lys Cys Leu Met Leu Ile Met Ala Leu Ala Ile Ile Asn Thr Val 1 5 10 15 Ser Ser (2) INFORMATION FOR SEQ ID NO: 12: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 16

amino acids (B) TYPE: amino acid (C) STRANDEDNESS: (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (ix) FEATURE: (A) NAME/KEY: Modified-site (D) OTHER INFORMATION: /product= "OTHER" /note= "Xaa = N-acetyl cysteine" (ix) FEATURE: (A) NAME/KEY: Modified-site (B) LOCATION: 16 (D) OTHER INFORMATION: /product= "OTHER" /note= "Xaa = valinamide" (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12 Xaa Asp Lys Ser Thr Leu Thr Asn Ala Tyr Val Asp Lys Ser Glu Xaa 1 5 10 15 (2) INFORMATION FOR SEQ ID NO: 13: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 23 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: DNA (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13 TGAAGTGTTT AATGCTGATC ATG 23 (2) INFORMATION FOR SEQ ID NO: 14: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 23 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: DNA (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14 CATTAGAGTA GCATAATGTA AGC 23 (2) INFORMATION FOR SEQ ID NO: 15: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 21 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: DNA (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15 TCCAAGTGCG AAAAATCGAC G 21 (2) INFORMATION FOR SEQ ID NO: 16: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: DNA (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16 GCTGATGGAG CAGGTACACC 20 (2) INFORMATION FOR SEQ ID NO: 17: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 327 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: cDNA (ix) FEATURE: (A) NAME/KEY: misc_feature

Other Reference Publication (19):
Tomchaney, A.P., Morris, J.P., Kang, S.H., and Duman, J.G., "Purification, composition, and physical properties of a thermal hysteresis `antifreeze` protein from larvae of the beetle, Tenebrio molitor", Biochem. 21: 716-721 (1982).

CLAIMS:

1. An isolated or recombinantly expressed antifreeze protein, said protein comprising the following properties:

(i) an apparent molecular weight of between about 5 and 20 kDa as determined by molecular exclusion chromatography;

(ii) a thermal hysteresis activity of greater than about 1.5.degree. C. at a concentration of about 1 mg/mL; and

(iii) (a) specific binding to an antibody raised against an antifreeze protein selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:3; and,

(b) encoded by a nucleic acid which specifically hybridizes to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1 under stringent conditions, wherein the stringent conditions include a wash step comprising a wash in 0.2.times.SSC at a temperature of about 65.degree. C. for about 15 minutes.

4. The isolated or recombinant antifreeze protein of claim 1, wherein the thermal hysteresis activity is greater than about 2.degree. C. at a concentration of about 1 mg/mL.

13. A liquid comprising a recombinant antifreeze protein comprising the following properties:

(i) an apparent molecular weight of between about 5 and 20 kDa as determined by molecular exclusion chromatography;

(ii) a thermal hysteresis activity of greater than about 1.5.degree. C. at a concentration of about 1 mg/mL; and

(iii) (a) specific binding to an antibody raised against an antifreeze protein selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:3; and,

(b) encoded by a nucleic acid which specifically hybridizes to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1 under stringent conditions, wherein the stringent conditions include a wash step comprising a wash in

0.2.times.SSC at a temperature of about 65.degree. C. for about 15 minutes.